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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 9/127, 9/107, 9/12, 31/685

A1

(11) International Publication Number: **WO 98/53800**

(43) International Publication Date: 3 December 1998 (03.12.98)

(21) International Application Number: PCT/US98/09903

(22) International Filing Date: 22 May 1998 (22.05.98)

(30) Priority Data:

60/048,013	29 May 1997 (29.05.97)	US
60/056,086	27 August 1997 (27.08.97)	US
60/064,495	5 November 1997 (05.11.97)	US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: COMPOSITIONS AND METHODS FOR PREVENTING ADHESION

(57) Abstract

A composition and method for preventing, reducing or eliminating fibrosis or adhesion formation or reformation in mammals following surgical, traumatic, organic or radiation injury to internal organs, peritoneal linings and other body spaces. The composition contains phospholipids that may be selected from the group consisting of phosphatidylglycerol, phosphatidylinositol or phosphatidylserine, phosphatidylcholine and phosphatidylethanolamine. The present invention also provides composition and methods to inhibit fibrosis, scarring and adhesion formation by both restoring natural lubrication, exhibiting natural tissue plasminogen activator properties and resistance to alpha phospholipase A2. Adhesion formation is inhibited by applying the composition to body cavities where tissue trauma has or is expected to occur.

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COMPOSITIONS AND METHODS FOR PREVENTING ADHESION

RELATED PATENT APPLICATIONS

This patent application is related to U.S. Provisional Patent Application Serial Nos. 60/048,013, filed May 29, 1997; 60/056,086, filed August 27, 1997; and 60/064,495, filed November 5, 1997, the disclosures of which are entirely incorporated herein by reference.

FIELD OF THE INVENTION

The present invention generally relates to compositions and methods for preventing or treating spontaneous or post-operative adhesion formation and fibrosis between biological tissue surfaces.

BACKGROUND OF THE INVENTION

The health problems associated with adhesion formation and fibrosis between biological tissue surfaces in mammals has been noted for generations. Postoperative adhesions are a major form of pathology in many humans and are a significant source of surgical complications, pain, mechanical bowel obstruction, female infertility, morbidity and even death. For instance, vascularized adhesions that form in the abdominal cavity typically result in pain, infertility and sometimes bowel obstruction resulting in death. Moreover, adhesion formation at the site of a laminectomy can lead to fibrosis and chronic pain in the spine. Further, post-operative adhesion formation at the site of joint surgery can result in limited range of motion and pain.

Typical causes of adhesion formation include rough surgical technique or intervention, fulguration, abrasion, localized infection, cautery, excessive bleeding, drying, anti-osmotic lavage, non-specific trauma, endometriosis, peritoneal dialysis, radiation and the like. The biological causes of adhesion formation, however, can be attributed to decreased lubrication, decreased tissue plasminogen activator and increased phospholipase activity. During the course of a long surgery, mopping and suctioning of the blood and fluids may wipe away or remove endogenous phospholipids and cause trauma and micro-abrasions that lead to drying out or injury

to the parietal and serosal peritoneum (decreased lubrication, decreased tissue plasminogen activator and increased phospholipase activity), thereby leading to adhesion formation.

For example, adhesions that form as a result of damage to peritoneal tissues (serosal or parietal) have been linked to ischemia in mesothelial tissues that normally produce phosphatidylcholine and tissue plasminogen activator (t-PA). Tissue plasminogen activator is a substance that plays a role in the lysis of thrombi. It is known that t-PA moderates fibrin deposition and formation by breaking down the fibrin deposits before they can become infiltrated with fibroblasts. The pathophysiological stimulus for the ischemia is a disruption of the intrinsic peritoneal fibrinolytic system, which both controls t-PA production and activates alpha-phospholipase A2. As part of the normal healing process, fibrin is deposited at the site of an injury and forms thin, filmy adhesions within 3 hours of injury but spontaneously resolve after about 3-5 days. In healthy peritoneal tissues, t-PA formation originates in the mesothelium to interact with the fibrin exudate. Deficiencies in normal t-PA activity can allow thin filmy adhesions to become thick, fibrous and vascularized. Activation of the normally present, but inactive, alpha-phospholipase-A2 can cause hydrolysis of the phospholipid lubricant and protective coating. Moreover, disruptions in the mesothelium often result in fibrosis, adhesion formation and/or pain. It appears that adhesions formed by this disruption or damage are not dependent on either the amount or type of trauma.

Various methods and compositions have been proposed for treating or preventing adhesion formation. For example, meticulous surgical technique for cutting away adhesions has been tried but with little success because ischemic tissue produced as a result of suturing, grafting and patching of the peritoneum is a strong stimulus to adhesion formation. In addition, patch grafts or barriers made of synthetic substances (Surgicel™, Interceed™ Seprafilm™ and Gore-Tex™), as well as natural membranes (amniotic membranes) have been used with limited success or abject failure. Other measures, such as lavages containing dextran, glucocorticosteriods, antihistamines, anti-prostaglandins, ibuprofen, calcium channel blockers, hyaluronidase and urokinase, have been tried, but with varying and unsatisfactory

results. Lavage infusion treatments using synthetic recombinant t-PA has been shown to be useful in reducing post-operative surgical adhesion formation, but synthetic recombinant t-PA is prohibitively expensive and may cause systemic side effects. It has been shown to interfere with other tissue healing so as to result in poor healing and weakened scars at anastomosis sites, which can result in rupture, spillage and peritonitis. The concentration of the agent required to prevent or modify the formation of abdominal adhesions causes a significant reduction in the wound hydroxyproline concentration during the early healing phase, indicating significant reduction of the collagen content of the scar with subsequent loss of wound tensile strength.

Other relatively unsuccessful efforts include heparin, dextran, steroids, and calcium channel blockers. For instance, heparin has been known to actively reduced adhesions in human and animal studies, however, the dosage required to reduce adhesion formation typically results in undesirable hemorrhagic diathesis. In addition, dextran has not satisfactorily demonstrated the ability to consistently decrease adhesion formation, and typically causes many side effects, including ascites, pleural effusion, edema of the legs and vulva and even anaphylactic shock. Further, while corticosteroids have been used to prevent adhesion formation, it is believed that peritoneal surgery initiates an inflammatory response that simply overwhelms the therapeutic response to corticosteroids at studied doses. When higher doses of corticosteroids are used, however, their effects on organ systems not involved with adhesion formation per se create other clinical complications and concerns. Moreover, because of the potential for high-dose glucocorticoids to cause immunosuppression and poor wound healing, such agents are not practical for use in surgical patients. Although calcium channel blockers seem to have a slight adhesion reduction capability, they are of limited value unless used as a synergist or adjunct with other drugs such as t-PA. Even a new FDA approved hyaluronate agent has been shown to only slightly decrease adhesion formation by as little as only 5.9%.

Ar'Rajab A, et al., "Phosphatidylcholine Prevents Postoperative Peritoneal Adhesions: An Experimental Study in the Rat," J. Surg. Res., 50:212-215 (1991) discloses the use of Lipostabil ™ (Natterman, GmbH, Cologne) in rats. Because

Ar'Rajab does not disclose the use of sonication treatment or emulsifying/dispersing agents, it appears that they apparently used Lipostabil™ in liquid form. Lipostabil™ is a non-aqueous extract of naturally occurring lecithin that contains various non-isomer specific phospholipids (i.e., in addition to phosphatidylcholine), free fatty acids, cholesterol, other chemicals and impurities. Although Ar'Rajab concludes that his results suggest the prevention of adhesion formation in rats, many of the tested animals experienced wound dehiscence, peritonitis and/or death. On the other hand, Bellón J.M., et al., "Effect of Phosphatidylcholine on the Process of Peritoneal Adhesion Following Implantation of a Polypropylene Mesh Prosthesis," Biomaterials, 17:1369-1372 (1996), represents an attempt to reproduce the test and data disclosed in Ar'Rajab using purified synthetic dipalmitoyl phosphatidylcholine (Sigma) without any sonication treatment or emulsifying/dispersing agents. Bellon discloses disappointing results. The present inventor has attempted to reproduce Ar'Rajab's results using synthetic D-alpha dipalmitoyl phosphatidylcholine in saline as the active ingredient and a normal saline solution as a control. As a result, the active ingredient was unable to be solubilized, even with prolonged shaking. Moreover, the relative success of dipalmitoyl phosphatidylcholine as an anti-adhesion agent was less than that seen with the normal saline solution used as the control.

Accordingly, there remains a need for better compositions and methods that are capable of preventing adhesion formation or fibrosis without the attendant disadvantages of conventional compositions and methods.

SUMMARY OF THE INVENTION

The present invention is based on the unexpected discovery that certain phospholipids and synthetic derivatives are useful for preventing adhesion formation. Accordingly, it is a primary object of the present invention to provide a composition and method for inhibiting and preventing the formation and/or reformation of adhesions or fibrosis in mammals. The inventive composition may comprise an admixture of isolated, exogenous, purified and/or synthetic phospholipids, preferably having a positive net charge.

Further, by applying a composition of exogenous, synthetic, positively charged, isomer specific, phospholipids (phosphatidylcholine and phosphatidylglycerol) to damaged tissue, natural organ tissue surfaces remain lubricated and tend not to become adherent to adjacent tissue. Moreover, the isomer-specific (D-isomer) inventive composition possesses both lubricating and properties resistant to alpha-phospholipase-A2. The D-isomer of Phosphatidylcholine may also have inherent t-PA properties without the presence of t-PA. The L-isomer is not resistant to alpha-phospholipase A2 and further more its degradation product can be lethal. In another aspect, the present invention comprises a method for preventing adhesions which comprises replacing or replenishing natural phospholipids normally found in the peritoneal endothelium and underlying mesothelium of damaged tissues via directly or indirectly administering the isomer-specific phospholipid compositions of the present invention via any suitable route, such as lavage, endoscopic administration or topical application.

Additional objects and attendant advantages of the present invention will be set forth, in part, in the description that follows, or may be learned from practicing or using the present invention. The objects and advantages may be realized and attained by means of the instrumentalities and combinations particularly pointed out in throughout this description and the appended claims. It is to be understood that the foregoing general description and the following detailed description are exemplary and explanatory only and are not to be viewed as being restrictive of the invention, as claimed.

DESCRIPTION OF PREFERRED EMBODIMENTS

Before proceeding with a description of the specific embodiments of the present invention, a number of terms will be defined. Throughout this specification, the term "site of surgical trauma" shall mean tissue that has been injured in any way including, without limitation, tissue sites that have undergone incision, excision, drying, suturing, fulguration, abrasion, cauterization, contusion, manipulation, laceration, anastomosis, curettage, orthopedic surgery, cardiovascular surgery, neurosurgery, plastic, reconstructive surgery and the like. The term shall also mean

tissue that is adjacent to the injured tissue. The term "site of other trauma" shall mean intra-pelvic implants of endometriosis of any type or location, as well as clubbing, occlusion or adherence of the fallopian tubes or other tissues inadvertently manipulated during surgery. The term "solution" shall mean aqueous solution, emulsion, micro-emulsion or any other liquid to which a specific phospholipid has been added. The term "gel" shall mean a hydrogel that is thermo-reversible or thermo-irreversible, ionic or nonionic, and that serves as a carrier for a solution, emulsion, micro-emulsion or any other liquid to which a specific phospholipid compound has been added.

Endogenous phospholipids have been to shown to have numerous purposes. One such purpose is to act as a surfactant that allows internal organs, such as the large and small bowel, omentum, uterus, tubes, ovaries, appendix, gall bladder and sigmoid colon to move freely relative to each other. In this way, the monolayer coating of phospholipids acts as a lubricant. In addition, certain endogenous phospholipids fill unoccupied spaces between internal organs and coat the outside surface of internal organs with a strongly adherent hydrophobic film.

In the peritoneum, phospholipids are synthesized in the mesothelium and secreted by the serosal or parietal peritoneal epithelium. Damage to these cell layers by trauma, infection, surgical intervention, peritonitis, endometriosis, radiation and the like can adversely affect the ability of the mesothelium to secrete phospholipids and t-PA. Alternatively, such damage can activate the alpha-phospholipase-A2 enzyme, thereby leading to additional adhesion formation. Moreover, phospholipids are capable of adhering to the serosal and parietal walls of the mesothelium and/or endothelium in a monolayer densely packed film (depending on the concentration). As they are composed of both hydrophobic and hydrophilic parts, it is believed that positively charged phospholipids adhere to the negatively charged peritoneum by their positively charged parts (strongly positive quaternary ammonium ions) in such a way that their hydrophobic parts would be orientated into the cavity. Strong side bonds (hydrogen bonding) then form between the adjacent molecules reinforcing the protective covering. These side bonds promote cohesion and "durability" of the adsorbed layer while orienting the hydrophobic components toward the cavity and

away from the peritoneal surface ("Boundary Layer Lubrication" theory). Moreover, because phospholipids do not interfere with the physiologic clotting cascade like t-PA or heparin, compositions of the present invention may be used without fear of intra-operative hemorrhaging.

In accordance with the principles of the present invention, replacement or replenishment of positively charged phospholipids during or following surgery or trauma to the peritoneum and mesothelium, no matter what origin, promotes normal lubrication and healing without adhesion formation. As an aid to understanding the present invention, but without being limited thereby, the mechanism of action for the compositions of the present invention is believed to be replacement of the surfactant/lubricate properties for specific naturally occurring phospholipids, followed by activation of t-PA activity and/or inactivation of alpha-phospholipase-A2 enzyme. It is believed that some positively charged phospholipids, especially phosphatidylcholine, have their own t-PA properties and that negatively charged phospholipids can have anti t-PA properties. In addition, that some fragmented phosphatidylcholines are biologically active and can destroy the activity of phospholipase A2, thereby indirectly increasing the level of t-PA through the receptor for platelet activating factor (PAF). Further, the D-isomer of phosphatidylcholine is resistant to alpha-phospholipase-A2.

Free zinc ions have been demonstrated to stabilize cell lysosomal membranes, which are restricted to the surface of the peritoneal membrane and decrease the amount of fibrin allowed to leak through cells and gather as exudate. Zinc also has the ability to directly inhibit the ability of phospholipase A2 enzymes to function. In order to make the composition iso-osmotic, a zinc salt may be added.

In a preferred embodiment of the present invention, positively charged exogenous phospholipids are formulated as an aqueous adhesion-preventative micro emulsion composition that is capable of providing a long lasting lubricating film and/or protective coating to damaged tissues at the site of surgical or other trauma. The invention restores or replenishes phospholipids and t-PA production/activity to damaged mesothelial cells while being resistant to alpha-phospholipase A2, which can cause hydrolysis of the existing or remaining phospholipid layer. Because

phospholipids are not water soluble, the composition must be manipulated to form an emulsion or micellular solution.

The compositions of the present invention may be used during or after all types of surgery in which adhesion formation can be a complication. The phospholipids used in the compositions of the present invention may be extracted or obtained from any suitable source, including, without limitation, lecithin, chicken eggs, and soybean materials. In a preferred embodiment, the compositions comprise an aqueous solution/emulsion of a derivative or analog, including enantiomers, diastereomers, hydrates, salts or solvents thereof, of a phospholipid selected from the group consisting of phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol, phosphatidylserine and phosphatidylethanolamine.

Certain negatively charged phospholipids, such as phosphatidylinositol and phosphatidylserine, contain indirect t-PA inhibiting properties. It is known that negatively charged phospholipids play an important regulatory role in controlling the fibrinolytic system by activating an inhibitor of t-PA (PAL-1), thereby decreasing t-PA production and increasing adhesion formation. While negatively charged phospholipids such as phosphatidylinositol and phosphatidylserine, may be used as surfactants or lubricants in accordance with the principles of the "boundary theory" of the present invention, i.e., lubrication, it is most preferred to use positively charged phospholipids because of their additional anti-adhesion properties. Further, while neutral phospholipids such as phosphatidylethanolamine may be used in accordance with the principles of the present invention, they are less preferred. It is noted that if naturally derived lecithin is used, it should be treated/prepared so that the amount or effect of negatively charged phospholipids contained therein is eliminated or substantially reduced and the amount of the D isomer form increased. With the use of lecithin, it is also preferred that other contaminants including, but not limited to, cholesterol and free fatty acids are eliminated.

Physically damaged peritoneal tissue undergoes hydrolysis of its phospholipid layer by activation of alpha phospholipase-A2 activity or release of additional lipase following tissue trauma. The lipase is resistant to the D-isomer of the alpha-phospholipid. The L-isomer alone, as is found in natural lecithin, is a

surfactant/lubricant, but it does not satisfactorily limit adhesions because it is not lipase resistant. While providing lubrication it may increase the mortality rate due to its degradation into lysophosphatidylcholine, which is toxic, and arachidonic acid.

In a further aspect of the present invention, the inventive composition may comprise a racemic isomeric mixture (50/50) of D- and L-forms of suitable phospholipids. But, a composition substantially containing positively charged D-isomer forms of a phospholipids is preferred over L-isomer forms, which appear to be vulnerable to the alpha phospholipase-A2 enzyme and lethal. Examples of preferred compositions include, without limitation, compositions that comprise about 0.001% to about 100% of D-alpha or D-L alpha-phosphatidylcholine and about 0.005% to about 50% of phosphatidylglycerol, more preferably, about 0.01% to about 50.0% of D-alpha-phosphatidylcholine and about 0.05% to about 50.0% of phosphatidylglycerol.

To prevent adhesion formation during laminectomy, nerve or tendon surgery the compositions of the present invention may be directly applied to the surgical field. In a preferred embodiment, the inventive composition may be intra-operatively administered to a wound to stabilize the peritoneal endothelium, protect the mesothelial cells and to prevent fibrin leaking and early adhesion formation.

During laparotomy, bowel packs and retractors are used which may inadvertently damage or dry other abdominal tissues not in the direct field of the intended procedure. In such an application, the inventive composition may be formulated with an irrigation solution, viscous or thixotropic solution, gel, foam ointment, emulsion or aerosol.

The compositions of the present invention may be introduced into the peritoneal cavity via endoscopic attachments in either a solution or gel form and be applied directly onto or directly adjacent to the diseased tissue, peritoneal, mesothelium etc. The inventive compositions may also be applied directly to adjacent tissue via the scope or its accessories. Adequate lavage and irrigation with compositions of the present invention will serve to re-moisten and coat tissues that may be either adjacent or distant, but damaged during this surgical procedure.

The compositions of the present invention may be administered by any convenient route or means to prevent the adhesion formation at sites of surgical or other trauma. Preferably, compositions of the present invention may be administered in any acceptable delivery formulation that is useful for maintaining local effective concentration levels of the phospholipids in the inventive compositions at the site of surgical or other trauma. In another preferred embodiment, the inventive compositions may be applied and replenished via an indwelling catheter such as a peritoneal catheter over a suitable amount of time (a few hours to a few days) to insure that an adhesion preventing amount of active ingredient stays within the body cavity. Examples of administration routes include, without limitation, oral, intravenous, direct topical, irrigation or lavage. Preferably, the inventive compositions are topically and non-systemically administered to the surface of damaged tissues via a single dose prior to closing the surgical field. More preferably, the inventive compositions are applied not only to the affected tissue, but also to locally adjacent or neighboring tissues, as well. If necessary, compositions of the present invention may also be proximally or distally applied relative to the site or trauma, e.g., intra-peritoneally, to allow natural migration of fluids to occur in response to peristaltic contractions of the intestines.

The inventive composition may be administered in an effective amount and for a sufficient period of time to inhibit the formation of surgical adhesions. Preferably, the compositions are administered before significant healing or adhesion formation has begun. In some cases, effective treatment of tissue adjacent to, or near, affected tissues may be obtained by topical administration of the phospholipid compositions via an endoscope such as a laparoscope, cystoscope, arthroscope, mediastinoscope, colonoscope, etc., in a solution or gel.

The inventive composition may be formulated in any suitable way using any suitable carrier, so long as the phospholipid-based formulation concentration is sufficient to prevent adhesion formation and/or replenish the body's own contribution of phospholipids. Preferred examples include, but are not limited to, a solution, gel, emulsion, micro-emulsion, salve, cream, micellular suspension, film, aerosol or isotonic irrigation or lavage solution, etc. Because neither lecithin nor its component

phospholipids are water-soluble, it must be treated, e.g., chemically, prior to being utilized in an aqueous vehicle. For instance, co-surfactants may be required to form a micro-emulsion. Other methods include, but are not limited to, sonication, homogenization or micelle formation.

In accordance with the present invention, dispersing agents may be added to form a true surfactant emulsion that easily froths, bubbles and/or foams. Homogenization, sonication or the addition of a spreading, wetting or triturating agent may be used to form the emulsion. The addition of such agents to phospholipid compositions for anti adhesion indications is considered to be a necessity that has heretofore been unknown or unrecognized.

Sterilization of the formulation may be accomplished using any conventional method including, without limitation, aseptic preservation, filtration, exposure to gamma radiation, autoclaving and the like.

In another preferred embodiment, the phospholipid compositions are formulated into a water-soluble gel and packaged in a tube, hypodermic syringe or other suitable device for direct or extruded delivery to damaged tissue. A gel can be constructed to provide sustained release of active ingredient by anyone skilled in the art. The gel may be thermo-reversible or thermo-irreversible and may also be ionic or nonionic. In the gel, phospholipids may be emulsified, homogenized, micellized or used in liposome form so as to be uniformly distributed in the gel. Generally, the viscosity of the gel should be pliant at body temperature and capable of coating traumatized tissues. Methods for incorporating phospholipid mixtures into gels, emulsion, solutions are well known to those skilled in the art. For instance, the mixture may be in the form of a liposome or micellular medicament or may be freeze dried so as to be reconstituted immediately prior to use. The exact mode of administration is not critical so long as the adhesion-preventative composition can be administered in direct or adjacent contact to the injured tissue to achieve the desired effect.

In accordance with the principles of the present invention, an effective dose for topically applying the inventive composition is normally expressed in terms of concentration of the drug in the carrier (% weight/volume) coupled with the number

of times per day the drug is applied. The effective dose for any given application will depend upon factors such as the nature of the phospholipid or phospholipid mixture used, nature of the vehicle or carrier, the nature and/or site of the tissue to be treated, the type of trauma or lesion and the mode of delivery, e.g., one-time delivery via a lavage or sustained release vehicle, continuous delivery via indwelling catheter, or direct topical application either manually or via an endoscopic device.

Regarding appropriate dosages, it is to be understood that no hard and fast rule can be provided that will apply in all cases. It is well within the ability of a person skilled in the art to determine the thresholds and therapeutic dosages and frequency at which the phospholipids should be administered for any given application or administration route. Important factors include the type and nature of the injured tissue and the extent of tissue injury. Preferred concentrations of the phospholipids will approximate the physiologic concentrations found at the site of healthy tissue (mesothelial tissue in the case of phosphatidylcholine or mature fetal lung tissue in the case of phosphatidylglycerol). For instance, an effective dose of a solution containing phosphatidylcholine, which replicates the physiologic concentration levels in the peritoneal cavity of the human animal, is at least about 1.0 mg/ml. Minimal concentrations exhibit anti-adhesion activity in most cases. Higher or lower amounts and concentrations of the phospholipids in the compositions of the present invention may be appropriately used as readily determined by one skilled in the art.

The composition and method of the present invention will be further illustrated in the following, non-limiting Examples. The Examples are illustrative of preferred embodiments only and do not limit the claimed invention regarding the materials, conditions, process parameters and the like recited herein. All amounts are in weight/volume percent unless otherwise noted.

EXAMPLE 1

An irrigation/lavage solution containing D-Alpha phosphatidylcholine dipalmitoyl and dispersing agents in an iso-osmotic and/or isotonic saline solution is sterilized and pH buffered with appropiate buffers to a physiologic pH of about 7.4. During a surgical operation, the solution is applied to the surgical field to keep

exposed tissues moist and rinse away blood, pus, fibrin deposits, etc. Prior to closing the surgical incision, a final irrigation with the solution is instilled into the surgical field and the surgical wound. It is noted that excess solution may or may not be removed prior to closing of the surgical incision. The solution is allowed to remain in the surgical field thereby providing a protective covering and lubricating agent to the exposed organs.

EXAMPLE 2

A gel, ointment or solution containing appropriately dispersed,emulsified, or micellized D-alpha phosphatidylcholine is directly or indirectly applied via an endoscope by extrusion or other methods during endoscopic surgery to areas affected directly or indirectly during the endoscopic surgery so as to provide a protective and lubricating covering to the affected tissues while contributing its anti-adhesion formation properties.

EXAMPLE 3

A gel, ointment, solution, foam, aerosol of other suitable method containing appropriately dispersed or emulsified D-Alpha Phosphatidylcholine is directly or indirectly applied by topical route to an open wound such as a of laminectomy surgery, ophthalmologic surgery, burn, incision, excision, etc. so as to provide a protective and lubricating covering to the affected tissues while contributing its anti adhesion formation properties.

EXAMPLE 4

A surfactant solution of Tyloxapol-saline (Composition A) was first prepared by dissolving 250 mg of Tyloxapol (Sigma Corporation) in 0.1 N sodium chloride to produce a total volume of 250 ml. Then, 200 mg of D-alpha-phosphatidylcholine dipalmitoyl (DPPC) (Sigma Corporation) and 45 mg of hexadecanol (Sigma) were combined in powder form and placed in a screw cap tube (Composition B). A 30 ml sample of Composition A was added to the powdery Composition B, warmed to 60° C and then vigorously mixed to obtain a uniformly milky suspension solution (adhesion preventative composition). Aliquots of 1.5 ml each (10-mg D-Alpha-DPPC) can be immediately used, for example, as a dosage for intra-peritoneal infusions. The

adhesion preventative composition can also be immediately frozen or freeze-dried for long-term storage using routine lyophilizing techniques. If the container is sealed while under vacuum, then the resulting lyophilized product can be stored in vials at room temperature in its powder form. For longer storage periods, the lyophilized product can be stored at 4° C until use, at which time, it must be reconstituted to its aqueous form prior to utilization.

EXAMPLE 5

A 250 ml aliquot of a first composition is prepared by mixing about 225 mg of NaCl, 250 mg (0.233 ml) of a surfactant (tyloxapol > 99% pure (Sigma)) and 250 cc of sterile water (Baxter). A 60 ml aliquot of a second composition is prepared by mixing about 90 mg of cetyl alcohol (Sigma >90% pure), which has been triturated to a fine dry power, and about 810 mg of D-alpha dipalmitoyl phosphatidylcholine (D-alpha-DPPC) powder (Sigma >98% pure). Then, the first and second compositions are combined to form about 60 ml of a final composition. Specifically, the final composition is prepared by mixing about 60 ml of the first composition with about 900 mg of the second composition at a temperature of about 70° C for about 20 minutes. The resultant 60 ml aliquot is equivalent to about 13.8 D-alpha-DPPC per ml, of which about 1.48 ml may be used for a 20 mg dose.

EXAMPLE 6

This Example shows a manner by which a stored, lyophilized, powdery, adhesion-preventative composition/surfactant, which is made according to Example 4, can be reconstituted prior to utilization. A dose of distilled or sterile water for injection USP was drawn into a syringe with a standard, sterile, hypodermic needle. The vial cap was penetrated with the needle so that the water was drawn into the hypodermic needle under the vacuum of the vial. When the water was introduced into the vial, the surfactant spontaneously dispersed into solution to form a milky colored adhesion-preventative aqueous composition, which may be used. A dose of 10-mg DPPC was used in rats as an intra peritoneal lavage to achieve desired results according to the principles of the present invention. For use in a freeze-dried form,

the surfactant solution was reconstituted prior to administration as a lavage for an affected peritoneal or denuded surgical surface. It must form a stable film that lowers surface tension to very low values (10 milliNewton/meter or less).

A relatively simple *in vitro* test has been devised to test for natural surfactants in amniotic fluid. *See* Clements, New England Journal of Medicine, Vol. 286, pages 1007-1081 (1972). This test is generally known as the "Shake Test." While relatively simple to apply, the Shake Test can be used to assay the desired properties required by the inventive surfactant composition. For example, a carefully mixed sample of the composition containing approximately 400 micrograms (about 25 micro liters) is placed in a 20-ml culture tube. Two (2) ml of normal saline is then added and the tube is tightly capped and immersed in a water bath for 5 minutes at 37° C to sufficiently equilibrate the temperature of the sample with the temperature of the bath. The tube is then shaken vigorously by hand for 15 seconds and replaced in the water bath to settle. The presence of copious foam at the meniscus will indicate that the surfactant had been adsorbed into the saline and had created a surface film thereon. If the bubbles are relatively small and remain for 15 minutes or more, then the test confirms that the surface film is stable and will maintain a low surface tension.

EXAMPLE 7

An adhesion-preventative composition was prepared containing 6.67 mg/ml of D-alpha-DPPC (greater than 98% pure, Sigma Corporation) and 1.5 mg/ml of hexadecanol (greater than 99% pure, Sigma Corporation) dispersed into 1 mg/ml of a surface active agent (Tyloxapol in 0.1 N Nalco, Sigma corporation), as described in Example 4. The adhesion-preventative composition lowered aqueous surface tension rapidly and produced a surface tension less than 10 milliNewtons per meter. (The surface films created by the adhesion-preventative composition were stable after more than eight days after undergoing the Shake Test described above in Example 6.) The resultant composition was then used in the following *in vivo* tests employing 30 Sprague-Dawley rats divided into 2 groups (Groups A and B). .

Groups A and B received a laparotomy incision under clean, anesthetized conditions. The cecum of each rat was grasped and vigorously abraded with a dry

sponge, then scraped with a scalpel. Five (5) drops of 95% ethyl alcohol were applied to the manipulated cecum to further enhance an inflammatory response.

Control Group A received 1.5 ml of normal saline irrigation prior to closure. Group B received a 1.5 ml irrigation of the composition of Example 4 (10 mg D-Alpha-DPPC). The laparotomy incision of both groups was closed with 4-0 chromic suture (Ethicon) and sterile skin staples. The animals in both Groups were maintained under routine conditions, e.g., fed copious food and water. There were no deaths in either group. At 8 days post-op, the test animals were sacrificed and necropsy was performed utilizing the grading criteria described in Table 1 below.

Table 1: Grading of Adhesions

Grade	Observed Gross Appearance
0	No adhesions
1	Single omental band or thin, filmy: (Mostly avascular; limited to one area; easily separable)
2	Two bands or adhesions which withstand traction
3	Dense adhesions involving the adjacent mesentery, intestinal or other intra-abdominal organs
4	Viscera directly attached to the abdominal wall

A statistical analysis of the adhesions scored between two groups (*see* Table 2) was performed using descriptive statistical analysis (Table 3). The results were subjected to the Wilcoxon-Mann-Whitney test for ordinal data. As a result, the post surgical adhesion formation was significantly lower in the test animals ($P<0.0002$).

Table 2: Gross Findings

(utilizing 10 mg dose of D-alpha-DPPC)

Group A (Control)		*Group B (Inventive Composition)*	
Rat No.	**Score**	**Rat No.**	**Score**
1	3	1	4
2	4	2	3
3	3	3	2
4	3	4	0
5	3	5	1
6	3	6	3
7	4	7	1
8	4	8	0
9	4	9	1
10	3	10	1
11	3	11	1
12	3	12	1
13	2	13	1
14	3	14	2
15	3	15	3

Table 3: Descriptive Statistical Analysis

Count	Mean	Std. dev.
15	3.2	0.560612
15	1.6	1.183216

Table 4. Results for the Wilcoxon-Mann-Whitney Test

Expectation Variance (corrected for ties)	17.5	98	461.4815

Centered reduced statistic used for Wilcoxon-Mann-Whitney test: 3.747302

Critical value under normality assumption for a significance level of 0.0100: 2.5758

Probability corresponding to this U statistic under normality assumption: 0.0002

Conclusion:

Using a 99.00% confidence range, one CANNOT say that the control and experimental groups come from the same population.

The foregoing description of preferred embodiments of the present invention have been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the invention to the precise forms disclosed. Obviously, many modifications and variations will be apparent to practitioners skilled in this art. Similarly, any process steps described might be interchangeable with other steps in order to achieve the same result. The embodiments were chosen and described to best explain the principles of the invention and its best mode practical application to thereby enable others skilled in this art to understand the invention for various embodiments and with various modifications as are suited to the particular uses contemplated. It is intended that the scope of the invention be defined by the claims appended hereto and their equivalents.

WHAT IS CLAIMED IS:

1. A composition for preventing the formation of adhesions in mammals, the composition comprising a phospholipid and a suitable carrier.

2. The composition of claim 1, wherein the phospholipid is treated for aqueous administration.

3. The composition of claim 1, further comprising an emulsifying agent.

4. The composition of claim 1, further comprising a dispersant.

5. The composition of claim 1, wherein the phospholipid is phosphatidylcholine.

6. The composition of claim 1, wherein the phospholipid is selected from the group consisting of phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine.

7. The composition of claim 1, wherein the phospholipid carries a net positive charge.

8. The composition of claim 1, wherein the phospholipid carries a net neutral charge.

9. The composition of claim 1, wherein the phospholipid carries a net negative charge.

10. The composition of claim 1, wherein the phospholipid is substantially pure and stereo specific.

11. The composition of claim 1, wherein the phospholipid comprises the D-isomer form.

12. The composition of claim 1, further comprising a member selected from the group consisting of an organic material, inorganic salt or free ions.

13. The composition of claim 1, further comprising zinc ions.

14. The composition of claim 1, wherein the carrier is selected from the group consisting of: an emulsion, a liposome complex, a micellular complex, spray, foam, gel, lavage, cream, ointment and a non-ionic or ionic solution.

15. The composition of claim 14, wherein the gel is bio-resorbable.

16. The composition of claim 14, wherein the gel is thermo-reversible.

17. The composition of claim 14, wherein the gel is non-thermo-reversible.

18. The composition of claim 14, where the phospholipid is present in an emulsion or solution in a concentration of between 0.001% and about 30%.

19. The composition of claim 1, further comprising a surfactant, dispersing agents and water.

20. The composition of claim 1, further comprising an isotonic irrigant.

21. The composition of claim 20, wherein the composition is iso-osmotic.

22. The composition of claim 21, further comprising a zinc salt.

23. The composition of claim 1, further comprising an alcohol.

24. A method for preventing adhesion formation in mammals, comprising: topically administering to an injured tissue surface a composition comprising a phospholipid and a carrier.

25. The method of claim 24, wherein the phospholipid is treated for aqueous administration.

26. The method of claim 24, wherein the composition further comprises an emulsifying agent.

27. The method of claim 24, wherein the composition further comprises a dispersant.

28. The method of claim 24, wherein the phospholipid is phosphatidylcholine.

29. The method of claim 24, wherein the phospholipid is selected from the group consisting of phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine.

30. The method of of claim 24, wherein the phospholipid carries a net positive charge.

31. The method of claim 24, wherein the phospholipid carries a net neutral charge.

32. The method of claim 24, wherein the phospholipid carries a net negative charge.

33. The method of claim 24, wherein the phospholipid is substantially pure and stereo specific.

34. The method of claim 24, wherein the phospholipid comprises the D-isomer form.

35. The method of claim 24, wherein the composition further comprises a member selected from the group consisting of an organic material, inorganic salt or free ions.

36. The method of claim 24, wherein the composition further comprises zinc ions.

37. The method of claim 24, wherein the carrier is selected from the group consisting of an emulsion, a liposome complex, a micellular complex, spray, foam, gel, lavage, cream, ointment and ionic or non-ionic solution.

38. The method of claim 37, wherein the gel is bio-resorbable.

39. The method of claim 37, wherein the gel is thermo-reversible.

40. The method of claim 37, wherein the gel is non-thermo-reversible.

41. The method of claim 37, wherein the phospholipid is present in an emulsion or solution in a concentration of about 0.001% and about 30%.

42. The method of claim 24, wherein the composition further comprises a surfactant and water.

43. The method of claim 24, wherein the composition further comprises an isotonic irrigant.

44. The method of claim 42, wherein the composition is iso-osmotic.

45. The composition of claim 43, wherein the composition further comprises a zinc salt.

46. The composition of claim 25, wherein the composition further comprises an alcohol.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/09903

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 9/127, 9/107, 9/12, 31/685
US CL : 424/450, 45; 514/78, 937

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/450, 45; 514/78, 937

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS:
Search terms: adhesion?, phospholipid?, liposome?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 5,464,942 A (SAKURAI et al) 07 November 1995, abstract, column 36, lines 34-67, Examples and claims.	1-7, 9-10, 12, 14, 19-21, 24-30, 32-33, 35, 37 & 42-44 --- 8, 11, 13, 15-18, 23, 31, 34, 36, 38-41 & 45-46

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 27 JULY 1998	Date of mailing of the international search report 18 SEP 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer GOLLAMUDI S KISHORE Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/09903

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,411,743 A (MOORE et al) 02 May 1995, abstract, columns 1-3, Examples and claims.	1-2, 4-5, 8, 10-12, 14, 20-21, 24-25, 27-28, 31, 33-35, 37, 41, 43 & 44
X -- Y	AR'RAJAB, A. et al. Phosphatidylcholine Prevents Postoperative Peritoneal Adhesions: An Experimental Study in the Rat. Journal of Surgical Research. 1991, Vol. 50, pages 212-215, especially page 212.	1, 2, 5, 8, 12, 14, 18, 20, 21, 24, 25, 28, 31, 35, 37, 41, 43 & 44. --- 3-4, 6-7, 9-11, 13, 15-17, 19, 23, 26-27, 29, , 30, 32-34, 36, 38-40, 42 & 45-45
X -- Y	WEINER, A. L. et al. Liposome-Collagen Gel Matrix: A Novel Sustained Drug Delivery System. Journal of Pharmaceutical Sciences. September 1985, Vol. 74, No. 9, pages 922-925, especially page 922.	1, 2, 4, 5, 8, 12, 14, 15, 17, 20 & 21 --- 3, 6-7, 9-11, 13, 16 & 18-19
X	EP 0 162 724 A2 (VESTAR RESEARCH INC.) 27 October 1985, abstract.	1, 2, 4, 5, 8, 12, 14, 15, 16, 20 & 21